Insulin Signaling Deficiency Produces Immobility in *Caenorhabditis elegans* That Models Diminished Motivation States in Man and Responds to Antidepressants

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Keywords

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Abstract

Defects in insulin signaling have been reported in schizophrenia and major depressive disorder, which also share certain negative symptoms such as avolition, anhedonia, and apathy. These symptoms reflect diminished motivational states, which have been modeled in rodents as increased immobility in the forced swimming test. We have discovered that loss-of-function mutations in the insulin receptor (*daf-2*) and syntaxin (*unc-64*) genes in *Caenorhabditis elegans*, brief food deprivation, and exposure to DMSO produce immobility and avolition in non-dauer adults. The animals remain responsive to external stimuli; however, they fail to forage and will remain in place for >12 days or until they die. Their immobility can be prevented with drugs used to treat depression and schizophrenia and that reduce immobility in the forced swimming test. This includes amitriptyline, amoxapine, clozapine, and olanzapine, but not benzodiazepines and haloperidol. Recovery experiments confirm that immo-

bility is induced and maintained by excessive signaling via serotonergic and muscarinic cholinergic pathways. The immobility response described here represents a potential protophenotype for avolition/anhedonia in man. This work may provide clues about why there is a significant increase in depression in patients with diabetes and suggest new therapeutic pathways for disorders featuring diminished motivation as a prominent symptom.

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Introduction

Defective insulin/IGF-1 signaling has been observed in major psychiatric illnesses, including schizophrenia and major depressive disorder (MDD). Interestingly, schizophrenia was formerly treated with insulin shock therapy [1]. More recent studies have documented abnormalities in glucose metabolism in schizophrenic patients [reviewed in 2] and revealed a deficit in IGF-1 along with concomitant insulin resistance in this population [3]. In a similar vein, patients with type 2 diabetes mellitus and insulin resistance show a 2- to 3-fold increase in the

prevalence of MDD compared to the general adult population or patients with other chronic diseases [4–6]. MDD and schizophrenia share an array of negative symptoms that are variously referred to as apathy, anhedonia, avolition, and social withdrawal [7, 8]. Taken together, these observations suggest there might be common origins for the shared symptomatic behavior. We reasoned that reduced insulin signaling could produce changes in motivational states that underlie normal affective function. This is consistent with emerging ideas about a possible role for insulin signaling in depression [9].

Many psychiatric illnesses show characteristic disturbances in reward and motivational systems [10]. In schizophrenia, avolition is considered to be an established endophenotype [11, 12], whereas in MDD, anhedonia – or diminished pleasure-seeking – is a candidate marker for the disease [13, 14]. Anhedonia is part of a larger negative symptom complex in depression that includes apathy. In fact, anhedonia and apathy both reflect reduced motivation to either experience pleasure or actively engage in life. Although avolition and apathy are terms that have been applied to differentially characterize symptoms of schizophrenia and depression, respectively, they actually describe the same phenomenon – diminished motivational states, which are common to various psychiatric disorders. Marin [15] and Starkstein and Leentjens [16] have defined disorders of diminished motivation as a decrease in goal-oriented thoughts and behavior in the absence of altered consciousness, attention deficits, language impairment, or sensorimotor loss.

Recently, we proposed the concept of “protophenotypes” – endophenotypes (disease markers) that are conserved across evolution – and demonstrated that altered social behavior in *Caenorhabditis elegans* is a suitable counterpart to asociality in man [17]. The validity of this line of reasoning is further supported by studies that investigate “emotion primitives,” or rudiments of human emotional behavior, in *Drosophila* and *C. elegans* [18]. Because motivation is essential to drive behavior in all animals – to locate food, avoid harm, and find mates – aspects of this system are likely to have been conserved through evolution and qualify as protophenotypes. Consequently, we hypothesized that knockdown of insulin/IGF-1 signaling in *C. elegans* might alter goal-oriented behavior and serve as a protophenotype for diminished motivational states in MDD and schizophrenia.

As reported here, animals with loss-of-function mutations in the *C. elegans* insulin receptor (*daf-2*) or syntaxin (*unc-64*) genes become immobile, but not unresponsive, when subjected to brief food deprivation in the presence

of dimethyl sulfoxide (DMSO). This immobility response is analogous to that observed in rodents in the forced swimming test, which is used to model despair in relation to affective disorders [19–21]. The goal of this work is to identify genetic and other factors that reduce fundamental, goal-oriented behavior and to identify pharmacological approaches to overcome the genetic defects. This work may lead to a better understanding of the molecular regulation of motivation and new therapeutic pathways for disorders featuring diminished motivation/avolition.

Materials and Methods

General Handling of C. elegans and the Strains Used

The *C. elegans* strains used for these experiments were obtained from the Caenorhabditis Genetics Center and include: N2 (wild type), DR1574 *daf-2(e1391)*, DR1565 *daf-2(m596)*, DR1942 *daf-2(e979)*, DR1568 *daf-2(e1371)*, CB169 *unc-31(e169)*, and CB246 *unc-64(e246)*. *C. elegans* was cultured at 15°C according to standard growth conditions described previously [17, 22]. For induction of immobility, the animals were shifted to 25°C (the restrictive temperature) overnight prior to use in the assay.

Induction of Immobility (Diminished Motivational State)

The loss-of-function mutations in insulin signaling are temperature sensitive and manifest at temperatures above 25°C. The animals were grown on 90-mm nematode growth medium (NGM) plates (15°C) with food and 5X peptone and were then shifted to 25°C overnight (16–18 h). They were maintained in a well-fed state with ample food. Next, the animals were transferred with a platinum wire pick to standard 60-mm NGM plates with food in the absence (control) or presence of DMSO (1% final concentration) for 90 min. We then placed them on 90-mm NGM plates without peptone or food and in the absence or presence of DMSO (1%), depending on the experiment. This protocol is depicted in Table 1.

To prevent transfer of bacteria, we selected animals that were off the bacteria or we removed some from the bacteria to a region of the plate without bacteria and allowed them to crawl for 5–10 s before moving them to plates without food. At various times after transfer, we observed the animals individually for 5 s and scored them as “spontaneously moving” if they traveled more than 2 head lengths (about half their body length) in either direction as described elsewhere [23]. All incubation steps and observations were performed at 25°C or room temperature, respectively.

Reversal of Immobility with Pharmacological Agents

The drugs and neurotransmitters used in these studies were obtained from Sigma-Aldrich (St. Louis, MO, USA) or Tocris Bioscience (Bristol, UK). Clonazepam and diazepam were gifts from Dr. Nicholas Goeders (LSU Health Sciences Center at Shreveport). Olanzapine, clozapine, haloperidol, clonazepam, diazepam, amoxapine, loxapine, amitriptyline, cyproheptadine, ritanerlin, and metergoline were dissolved in DMSO. The volume of drug-DMSO was taken into account when setting up the test plates containing DMSO, i.e., the total amount of DMSO was 1%. Serotonin, dopa-

Table 1. Time course of immobility response and effect of octanol

General protocol						
NGM (5X peptone) plate plus food, 15°C						
↓						
NGM (5X peptone) plate plus food, 25°C overnight (16–18 h)						
↓						
NGM plate plus food (± DMSO + solvent or drug), 25°C for 90 min						
↓						
NGM plate minus food (± DMSO + solvent or drug), 25°C for the observation period						
	Moving/total					
	1 h	3 h	24 h	48 h	72 h	96 h
<i>daf-2(e1391)</i> DMSO, no food	5/38					
<i>daf-2(e1391)</i> DMSO, no food + octanol challenge	30/34					
N2 DMSO, no food	25/26	19/20	8/8	6/7	3/3	0 ^a
<i>daf-2(e1391)</i> DMSO, no food	3/22	4/20	0/20	1/22	1/22	0/21
<i>unc-64(e246)</i> DMSO, no food	4/24	2/25	3/25	1/25	1/22	0/22

Animals (25–40 per plate) were transferred to bacteria-free NGM plates containing DMSO, as shown at the top of the table. For the octanol experiments, we dipped a bristle from a horsehair paintbrush in octanol, held this in front of the nose of the immobile animals, and tallied whether they backed away as expected. For the time course experiments, we periodically assessed spontaneous movement of animals over 96 h. At all time points, the animals still escaped normally in response to tail touch. The steady decline in numbers in the control (N2) group reflected animals that attempted to leave the no-food environment, crawled up the side of the plate, and desiccated off the agar. This represents a typical response of *Caenorhabditis elegans* strains with a normal drive for food seeking. NGM, nematode growth medium. ^a No animals were left on the plate at this time point.

mine, octopamine, tyramine, carbachol, telenzepine, pirenzepine, and atropine were dissolved in water to obtain solubility.

The drugs and DMSO were introduced onto plates (60-mm NGM-peptone plus food and 90-mm NGM with no peptone) to give the final desired concentrations based on the total agar plus drug-DMSO volumes. The neurotransmitter and drug concentrations evaluated in these experiments were based on either standard values from the literature or levels found effective for other drugs of the same general class [24, 25]. The drug and control plates were allowed to dry and equilibrate for 2–3 h prior to use. The animals were transferred to the drug or control plates and incubated at 25°C for 90 min. Then, they were placed on 90-mm NGM plates without food, but with drug-DMSO or dilute acetic acid-DMSO (control) and evaluated for spontaneous movement after 30 and 60 min on the plates as above. Typically, 25–40 animals were examined in each experiment, with additional repetitions for positive drugs.

Recovery and Blockade of the Recovery Response

To study recovery after induction of immobility, the animals were first transferred to plates with food and DMSO for 90 min and then moved to plates with 1% DMSO without food to induce immobility. After 2 h, the immobile animals were transferred back to the NGM plates with a spot of bacteria in the middle and containing no DMSO (control), DMSO, or drug (and DMSO) at the desired concentration. The animals were placed about one-third

of the way from the edge of the plate to the bacterial lawn. At 30 and 60 min, the number of animals on the lawn was counted, and any sluggishness or unresponsiveness to touch was noted. Movement to food was regarded as successful recovery from the diminished motivational state.

Statistical Analysis

To measure the effect of a treatment on movement, we counted the number of animals that were moving and the total number on the plate, or the number of animals on food in the recovery assay versus the total on the plate. These measures, e.g., moving versus not moving, are nonparametric. Therefore, we used a χ^2 test to evaluate whether differences between the treatment groups and the controls were statistically significant. We have previously employed the same statistical approach for the analysis of spontaneous movement in other strains [23]. The χ^2 method does not generate standard deviations. To give a sense for variability in the data, we calculated 95% confidence intervals for 6 representative sets of data. These values averaged 29% and had a range from 15 to 40%. This means that to be significantly different ($p < 0.05$) from the control group, the percentage of animals moving in the drug treatment groups typically had to be greater than ~30–35% when compared to the average number of animals moving in the control conditions, which was 9.6% (calculated from 24 experiments).

Results

Optimization of Conditions to Produce Immobility

C. elegans has a single receptor, DAF-2, for its ~40 insulin/IGF-1 proteins. Loss-of-function mutations in the *daf-2* gene are associated with constitutive dauer formation (a hibernation-like state of diapause) [26], defects in associative learning [27], and failure to recover pharyngeal pumping during starvation [28]. The latter finding was the starting point for the current studies. We sought to optimize conditions for inducing immobility in *C. elegans*, including genetic background and environmental influences. Pilot experiments revealed three requirements for inducing long-term immobility: (1) decreased DAF-2 function, (2) $\geq 0.8\%$ DMSO, and (3) food deprivation. These conditions led to rapid (<15–20 min off food) reduction in movement and immobility. DMSO was not harmful at the concentrations used in these experiments – chronic exposure to 1% DMSO actually extends the life span [29]. The state of immobility was easily reversible by re-exposure to food or removal of DMSO (see below). For the work reported here, we used young adult animals because we wanted to avoid dauer formation and developmental influences.

Genetic Analysis of Immobility Induction

With the optimized conditions, we compared the behavior of wild-type N2 controls with animals bearing *daf-2* alleles with varying degrees of temperature-induced deficits. Elevated temperature had no effect on the movement of N2 animals on or off food, in the presence or absence of DMSO (Fig. 1a, b). By contrast, temperature and food deprivation modestly decreased spontaneous movement in *daf-2(e1391)* animals. However, the combination of elevated temperature, food deprivation, and 1% DMSO drastically reduced spontaneous movement in this strain (Fig. 1a, b). Elevated temperature did not alter movement via developmental effects of *daf-2* loss of function, because the exposure was fairly brief (~16–18 h) and occurred after larval maturation to adulthood was already complete. This does not appear to be an adult dauer-like phenotype, because muscarinic agonists normally promote dauer recovery [30], whereas the immobile state is maintained by excessive muscarinic signaling (see below).

The animals were not paralyzed by these conditions. They remained fully responsive to touch on the tail with a wire pick and their escape movement was normal (Fig. 1b). In addition, they remained responsive to sensory cues such as octanol, which produces an avoidance response. Immobilized animals with the *daf-2(e1391)*

allele responded normally to octanol by rapid backing (Table 1), which suggested that their sensory function was intact. The animals remained in the immobile state for 96 h and longer (Table 1), even though during that time they occasionally moved for a few seconds before becoming immobile again. Many remained in the same location on the plate for the duration of the observation period and began to die in place after 12–13 days without food (data not shown). Thus, the animals were fully capable of moving and sensing external cues; however, they failed to show normal motivation to search for food or leave a low-nutrient environment.

By contrast, under identical conditions, we saw no effect on *daf-2(m596)* animals (Fig. 1c). Gems et al. [31] classified this *daf-2* allele as 2A, which is less severe than the class 2D allele of *e1391*, based on their different phenotypes including dauer formation, life span, L1 arrest, and brood size. Although not shown, animals with the class 1A allele *e1371* were not immobilized by these conditions, whereas the more severe allele *e979* produced similar outcomes to *e1391*. Therefore, the severity of the deficits associated with *daf-2* alleles with respect to the above phenotypes determined the degree of immobility in our behavioral assay. Unfortunately, the redundancy and number of insulin molecules produced in *C. elegans* restricted our ability to determine the effects of genetic mutations in the ligand for DAF-2.

Two genes, *unc-64* and *unc-31*, regulate insulin secretion in *C. elegans* [32], control dauer formation at the restrictive temperature of 27°C [33], and, in the case of *unc-64*, affect recovery of pharyngeal pumping during starvation, which requires insulin signaling [28]. UNC-64 (syntaxin) is involved in release from both dense-core vesicles and synaptic vesicles, whereas UNC-31 (Ca⁺⁺-dependent activator protein for secretion, CAPS) mainly regulates release from dense-core vesicles. Therefore, we evaluated animals with loss-of-function mutations in these genes for induction of immobility. Animals with the *unc-64(e246)* allele showed high levels of immobility very similar to *daf-2(e1391)* (Fig. 1c). Once again, the animals still responded to touch, indicating that they were not paralyzed, and they remained immobile for 96 h (Table 1). The *unc-31(e169)* animals moved at a lower basal rate but were not adversely affected by DMSO and food deprivation. In fact, their spontaneous movement increased with short-term food deprivation and DMSO compared to their behavior on food – the opposite of what was observed in *daf-2(e1391)* and *unc-64(e246)* animals. Greater movement of *unc-31* strains off food is an established phenotype of mutant animals. Despite near-

Fig. 1. Characterization of the immobility response of *daf-2(e1391)* animals. **a** We compared the spontaneous movement of wild-type N2 and *daf-2(e1391)* animals grown at 15 or 25°C under different conditions: food, food plus DMSO, no food, and no food plus DMSO, as indicated in the figure. A χ^2 analysis revealed significant differences from the appropriate control group (food with no DMSO or food plus DMSO), as noted with the asterisks: ** $p < 0.01$. **b** Still frames from video clips revealing spontaneous movement (upper two rows) or locomotion in response to tail touch (bottom row). The time elapsed in seconds is shown at the lower right-hand corner of each frame. The nose moved slightly from frame 1 to frame 2 of the *daf-2(e1391)* panel in the middle, otherwise the animal remained in the same place for the entire 15-s video, which is characteristic of this strain. The normal sinusoidal movements in response to touch continued well beyond the frames shown in the bottom row. **c** We evaluated 3 mutant strains to determine if they became immobile in response to brief food deprivation plus DMSO exposure. We found significant differences (** $p < 0.01$) when comparing *unc-64(e246)* animals off food plus DMSO with those on food plus DMSO, *unc-31(e169)* animals off food with those on food, and *unc-31(e169)* animals off food plus DMSO with those on food plus DMSO.

ly identical phenotypes otherwise including sluggish movement at 15°C, *unc-31(e169)* animals clearly behaved differently from *unc-64(e246)* animals in the immobility assay. Cai et al. [34] reported that genetic interactions with *ida-1* (IA-2, a protein tyrosine phosphatase-like receptor) produced opposite effects in *unc-31* (suppression) versus *unc-64* animals (enhancement) on dauer formation and aldicarb sensitivity. This confirms the independent functions of these two gene products, despite direct interactions between them, and may be related to the differences observed here. Together with the differential responses of the *daf-2* alleles, the data

suggest a richness and subtlety in the genetic control of the response.

Pharmacological Reversal of Immobility

We hypothesized that it should be possible to overcome the induction of immobility with the right pharmacological agent(s). We evaluated various neurotransmitters for their effects on immobility in *daf-2(e1391)* and *unc-64(e246)* animals. At concentrations that produced physiological responses such as egg laying and altered foraging, dopamine, octopamine, and tyramine did not affect immobility in these two strains (Fig. 2a). Serotonin

Fig. 2. Reversal of immobility with pharmacological agents. **a** Dopamine (DA), serotonin (5-HT), octopamine (OCT), tyramine (TYR), and carbachol (Carb) were evaluated for their effects on spontaneous movement in the immobility assay at the concentrations shown in the graph. Zeroes are included in this figure to indicate results where columns are lacking. **b** The benzodiazepines clonazepam (CZPM) and diazepam (DZPM) and the antipsychotic drugs clozapine (Cloz), olanzapine (Olan), haloperidol (Halo), and sulpiride (Sulp) were tested for their effectiveness in reducing immobility in the movement assay. They were evaluated at several

concentrations, and the results depicted here were obtained with 160 μ M. Significant differences from the control (DMSO) group are indicated with asterisks (** $p < 0.01$) in this figure. **c** The antidepressant drugs amitriptyline (Ami), amoxapine (Amox), fluoxetine (Fluox), and trazodone (Traz) and the atypical antipsychotic loxapine (Lox) were tested for their ability to correct spontaneous movement. The data depicted here were obtained with a drug concentration of 160 μ M. Significant differences are indicated by asterisks (** $p < 0.01$).

Fig. 3. Pharmacological basis for reversal of immobility. **a** The serotonergic (5-HT) antagonists ritanserin (Ritan), cyproheptadine (Cypro), and metergoline (Meter) reduced immobility in the spontaneous movement assay. The data shown here were obtained with 160 μ M drug. **b** The nonselective muscarinic cholinergic (mACh) antagonist atropine and the M_1 receptor-selective antagonists pirenzepine (Pirenz) and telenzepine (Telenz) were evaluated at final drug concentrations of 2 mM (atropine) and 160 μ M (pirenzepine and telenzepine). Significant stimulation of spontaneous movement in comparison to the DMSO control is indicated with asterisks (** $p < 0.01$).

and the cholinergic agonist carbachol also failed to stimulate spontaneous movement in the assay and appeared to further diminish basal activity (see below).

We initially thought our model of immobility might be a protophenotype for catatonia. Therefore, we tested drugs known to treat this condition, i.e., benzodiazepines and the second-generation antipsychotics clozapine and olanzapine. As seen in Figure 2b, clonazepam and diazepam did not correct the immobility of *daf-2(e1391)* and *unc-64(e246)* animals. At concentrations above 250 μ M, these drugs began to impair responsiveness of the animals. By contrast, clozapine and olanzapine both partially restored spontaneous movement in the assay (Fig. 2b). However, antipsychotic drugs highly selective for D_2 dopamine receptors (haloperidol and sulpiride) failed to reverse immobility (Fig. 2b), suggesting that the effects of clozapine and olanzapine were not mediated by dopamine receptors.

Because reduction of immobility in the forced swimming test is interpreted as an antidepressant effect in rodents, we speculated that established antidepressant drugs might likewise overcome immobility in our system. We evaluated several older tricyclic drugs, fluoxetine (the

original serotonin-selective reuptake inhibitor) and trazodone (a mixed 5-HT_{2A/2C} antagonist and serotonin-selective reuptake inhibitor). Amoxapine, amitriptyline, loxapine (methylated amoxapine, an atypical antipsychotic), and imipramine (data not shown) all produced a dramatic increase in spontaneous movement during food deprivation on DMSO (Fig. 2c). The locomotion was essentially normal, with periods of traveling interspersed with sharp turns and reversals. By contrast, fluoxetine and trazodone failed to reduce immobility (Fig. 2c). Many additional drugs had no effect on spontaneous movement, including nicotine, caffeine, flunarizine, buspirone, diltiazem, and nifedipine (data not shown), which attests to the selectivity of the effects. Furthermore, drugs (e.g., clozapine, loxapine, amitriptyline, atropine, metergoline, ritanserin, and telenzepine) that stimulated spontaneous movement in the immobility assay in the mutant strains did not affect movement in N2 animals.

Serotonergic and Cholinergic Antagonists Reduce Immobility

Because the serotonergic antagonists (clozapine and olanzapine) and muscarinic cholinergic inhibitors (tri-

Fig. 4. Inhibition of recovery with serotonin (5-HT) and the muscarinic agonist xanomeline (XNM). Animals previously immobilized by DMSO and food deprivation were transferred near the edge of plates with a central lawn of bacteria in the absence or presence of DMSO, as indicated. After 1 h, the number of animals that reached food was counted. The presence of serotonin or xanomeline (at the concentrations listed) significantly reduced recovery, as shown by the asterisks: * $p < 0.05$, ** $p < 0.01$.

cyclic antidepressants) reduced immobility, we explored the role of serotonin and acetylcholine in the response. Furthermore, serotonin and carbachol appeared qualitatively to cause sluggishness in the mutant animals. Several serotonergic antagonists, including ritanserin (5-HT_{2/1C}), cyproheptadine (5-HT_{2/1C}), and metergoline (5-HT_{2/1D/7}), largely prevented the onset of immobility caused by food deprivation/DMSO (Fig. 3a) and improved the foraging behavior of both strains.

To evaluate the role of acetylcholine, we studied the effects of the nonselective muscarinic antagonist atropine and the M₁ receptor-selective antagonists pirenzepine and telenzepine. The muscarinic agents clearly stimulated greater spontaneous movement in the assay (Fig. 3b), although they were generally less efficacious than the tricyclic antidepressants. These data confirm that acetylcholine, like serotonin, is somehow involved in the suppression of spontaneous movement observed in our system and implicates muscarinic receptors as a major target.

Recovery from Immobility

In pilot studies, we found that the immobility induced by food deprivation/DMSO exposure was rapidly reversed by reintroducing animals to plates with food in either the absence or presence of DMSO. Therefore, we sought to test whether serotonin and the M₁ receptor-selective drug xanomeline prevented this recovery response. Animals that were immobile after 2 h on bacteria-free plates with DMSO were transferred to the outer edges of plates with a spot of bacteria in the center, and after 60 min the number of animals on food in each group was counted. With both mutant strains, recovery was significantly reduced in the presence of serotonin or xanomeline (Fig. 4). Animals that failed to reach the bacterial lawn tended to be immobile, although they were still respon-

sive to touch. The results confirm that high levels of serotonergic and cholinergic signaling contribute to the induction/maintenance of immobility.

Discussion

Here, we describe the characterization of a novel immobility response produced as a consequence of genetic loss of function in insulin receptor signaling and acute food deprivation in the presence of DMSO. The immobility is not due to paralysis, sensory disturbance, or defective neuromuscular function, because the animals still respond normally to touch or aversive chemicals. Rather, the immobile state appears to reflect diminished motivation and failure to initiate goal-directed behavior – food-seeking and foraging. Perhaps, the most extraordinary aspect of this response is the observation that animals will remain in essentially the same place on plates without bacteria for >96 h, which is contrary to normal survival imperatives aimed at finding food. They will actually perish in place rather than searching for food or trying to escape.

Immobile states in vertebrates can be induced by stress (forced swimming) or acute restraint (tonic immobility and death feigning) and are considered models for MDD [19, 21] and catatonia [35, 36], respectively. Animals in these states are still responsive to their environment. From the current work, we extend the induction of responsive immobility to the invertebrate *C. elegans*. A common thread among these various states is a striking reduction in goal-directed behavior or internal drive. This might also reflect an adaptive response aimed at matching the behavioral output with expected rewards (risk-benefit analysis) or conserving efforts when reward

is unlikely – a strategy that predisposes to avolition and apathy in the context of affective disorders.

Diminished motivation can present as a stand-alone syndrome in man [10, 15], but also gives rise to the main negative symptoms of MDD (anhedonia, apathy) and schizophrenia (avolition, social withdrawal). These symptoms are treated, to varying degrees, with antidepressant drugs [37, 38], second-generation antipsychotics including clozapine and olanzapine [39–41], and muscarinic antagonists [42, 43]. The same drugs have been used to reduce immobility times in the forced swimming test [20, 44–46], and, as shown here, they significantly reduce immobility in *C. elegans*. Taken together, these observations suggest that our immobility model of diminished motivation is a suitable protophenotype [defined in 17] of established endophenotypes relevant to MDD and schizophrenia.

Induction of immobility requires a deficiency in insulin signaling, DMSO, and acute food deprivation. The precise role of DMSO is not known. Several recognized effects of this agent may be relevant: (1) DMSO inhibits binding of insulin to its receptor [47]; (2) it increases quantal synaptic output at the neuromuscular junction [48]; and (3) it directly inhibits acetylcholinesterase [49]. Of course, other explanations are possible. As to the mechanisms related to acute food deprivation, Wakabayashi et al. [50] have previously shown that serotonin is released during food deprivation and regulates foraging, whereas You et al. [51] found that brief starvation activates muscarinic cholinergic signaling in *C. elegans*. In the context of insulin receptor defects and DMSO, acute food deprivation may stimulate excessive release or signaling via these two neurotransmitters. This would explain why serotonergic and muscarinic cholinergic antagonists restore normal movement in our system. Because loss of function of syntaxin in *unc-64(e246)* animals mimics the effects of the *daf-2(e1391)* allele (albeit slightly weaker), we imagine that diminished syntaxin activity mainly reduces insulin release – a known function of this protein [32].

Both *daf-2(e1391)* and *unc-64(e246)* mutations prevent insulin-mediated recovery of pharyngeal pumping during prolonged (24- to 48-h) food deprivation. To account for these findings, we [28] previously proposed that the major signaling molecule Akt downstream of the insulin receptor regulates via phosphorylation a variety of ion channels, including voltage-gated Ca^{++} channels and the ketamine target UNC-68 (ryanodine receptor) [52].

The central role of insulin receptor signaling in regulating goal-directed behavior in *C. elegans* is intriguing, because it may provide clues about why there is a 2- to

3-fold increase in MDD among patients with diabetes [4, 5]. Insulin resistance is higher among depressed patients even in the absence of diabetes [53, 54], and apathy/anhedonia are common symptoms in diabetic patients [55, 56]. Furthermore, diabetic mice display greater immobility in the forced swimming test, and this is corrected by insulin administration [57]. Based on this apparent conservation of function, we speculate that insulin regulates motivation across species.

The findings reported here with serotonergic and muscarinic antagonists and inhibition of recovery with serotonin and xanomeline confirm the involvement of these pathways in the regulation of motivation. Intriguingly, cyproheptadine, ritanserin, metergoline, and scopolamine have all been reported to treat depression [42, 43, 58–61], whereas atropine has antidepressant effects in the forced swimming test [20]. There is a notable connection between muscarinic activity and insulin, not only in our system but also involving insulin regulation of muscarinic signaling in GABAergic neurons of the prefrontal cortex [62] – a site of scopolamine's antidepressant action [43].

Because well-established drugs that are used in primary (amitriptyline, amoxapine) or adjunctive therapies (clozapine, olanzapine) for MDD or psychotic depression restore normal goal-directed behavior in our system, we wonder if positive effects in the immobility assay might predict antidepressant activity in man. Although fluoxetine and trazodone failed to reduce immobility in our system, results with these drugs are also negative or mixed in the forced swimming test [63, 64]. Furthermore, this effort may reveal molecular mechanisms that contribute to diminished motivational states and are shared across evolution. Research with *C. elegans* has provided new clues about pathogenic processes involved in human neurodegenerative disorders, including Alzheimer and Parkinson disease [65, 66]. Perhaps, this simple organism can also yield significant insights into human psychiatric illnesses characterized by diminished motivation and avolition/anhedonia.

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Statement of Ethics

These studies were conducted according to general laboratory ethical guidelines. No human or animal use protocols were required for this work.

Disclosure Statement

None of the authors have any conflicts of interest to declare.

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